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Changes in the Protein Composition of Human Saliva Associated with Model Psychological and Emotional Stress

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Abstract—The usefulness of individual salivary protein spectra as indicators of resistance to stress is discussed. Analyses of total saliva performed before and after exposure to model stress showed that individual psychological and autonomic reactions correlated with specific changes in the concentrations of certain salivary proteins. Therefore, such an analysis could be useful for the assessment of individual resistance to stress.

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INTRODUCTION

Human saliva is known as a unique object of fundamental and applied medical research and diagnostic tests [1, 2].

Correlations between various physiological disorders and the functional activity of the salivary glands have led some researchers to consider them to be a mirror of disease [3]. Similarly, we regard saliva (especially the protein composition of the pooled secretion of all salivary glands) as a reflection of the subject's psychophysiological state [4–6]. This agrees well with data of other researchers that show psychogenic and emotional factors to be regulators of its biochemical composition [7–11].

Analysis of the physiological effects of stress-inducing factors is one of the most interesting fields of psychophysiological research. Considered in this context, psychological stress, whose manifestations are specifically determined by the subject's personality and individual psychological and emotional characteristics, can be associated with physiological reorganization, including the functional reorganization of the salivary glands.

With this in mind, we made an attempt to find correlations between the electrophoretic spectra of salivary proteins and psychological and emotional reactions of subjects exposed to model stress. We collected pooled samples of saliva and prepared them for electrophoresis; performed the electrophoretic separation of salivary proteins and quantitative densitometry of electrophoretograms; and detected and quantitatively analyzed the correlations between biochemical data and specific psychological reactions to stress, i.e., between biochemical parameters and psychological and autonomic (tachographic) characteristics of the subjects examined.

METHODS

The study was performed on 24 healthy 18- to 25-year-old male volunteers.

According to the current regulations (Bioethics Convention, Strasbourg, 1997), all the subjects were informed about the nature of the study [12]. The safety of all the tests and noninvasive manipulations was guaranteed.

To achieve the main goal of our study, we analyzed correlations between electrophoretic spectra of salivary proteins, on the one hand, and a combination of some psychological and autonomic characteristics of the subjects before and after model psychological and emotional stress (PES), on the other hand. This stress was induced by Sternberg's method of additive factors and the model of recognition of visual stimuli [13].

Tests for the subject's basic and current personal psychological characteristics most sensitive to stress [14, 15] were performed immediately before and after the model psychological stress tests, in parallel with registration of 5-min tachograms and collection of saliva samples (100–200 μl). The entire experiment lasted for 35–40 min. After the experiment, to eliminate the consequences of the model psychological and emotional stress, the essence of the study was explained to each subject.

The autonomic status was monitored cardiointervalographically with a VNS-Spektr computerized apparatus (Neurosoft, Ivanovo, Russia), which digitized the signals from ECGs (recorded in the first standard lead) and plotted them as tachograms. Initial records were analyzed visually, and interferences (such as extrasystoles) were removed according to standard algorithms [14].

The following parameters were calculated for the dynamic series of cardiac intervals recorded with cardiointervalograms: the heart rate (HR, beats/min), the standard deviation of successive RR intervals (SDNN, ms), the root-mean-square of successive differences between RR intervals (RMSSD), the percentage of successive RR interval differences above 50 ms (pNN_{50}, %), the mode (Mo, ms), the amplitude of the mode (AMo, %), the coefficient of variation (CV, %), the variation range of cardiac intervals (ΔX, ms), and the tension index (TI, arb. units). These abbreviations correspond to international standards for the assessment of heart rate variability (HRV) [14, 16]. The spectral analysis of the HRV provided the following frequency-related parameters: the total power of the spectrum (TP); the powers of the high-, low-, and very low frequency ranges (HF, 0.16–0.4 Hz; LF, 0.05–0.15 Hz; and VLF, <0.05 Hz, respectively); their percentages (HF%, LF%, and VLF%, respectively); and the LF/HF coefficient, which reflects the balance between sympathetic and parasympathetic regulatory effects on the heart.

To assess the stable individual traits of the personality and its current functional state, we used the following methods of psychological diagnosis localized in Russia by A.B. Leonova and her colleagues at the Laboratory of Occupational Psychology of Moscow State University [17, 18].

(1) Spielberger's state–trait anxiety inventory, which estimates personal anxiety, anger, and depression as stable characteristics of the personality. It also estimates situational anxiety (SAn) and situational anger (SAg), with subscales that reflect the emotional experience of anger (SAg/e) and the verbal and physical expressions of anger (SAg/v and SAg/ph, respectively). In addition, the test estimates situational depression (SD) with two subscales for dominant euthymia or dysthymic states (SD/eu and SD/dys, respectively).

(2) The scale of differential emotions based on Izard's theory of differential emotions [17]. This test yields a snapshot of ten current emotions related to the subject's personal assessment of the actual situation, including the indices of positive and acute negative emotions (PEM and NEM; they reflect the general levels of positive and negative assessments, respectively), as well as the index of anxious and depressive emotions (ADEM), which reflects relatively stable individual emotions of this kind.

(3) The method based on the "scale of states," which assesses the current levels of subjective comfort and discomfort [17].

The collected saliva was centrifuged for 10 min at 10000 *g* and stored at –20°C.

To denature salivary proteins, each sample was diluted (2 : 1, vol/vol) with a buffer solution (100 mM Tris (pH 7.5), 7% sodium dodecyl sulfate, 2% mercaptoethanol, 0.02% bromophenol blue, and 20% glycerol). The mixture was incubated for 10 min at 20°C, and 20 μl were applied onto a 12% polyacrylamide gel ($10 \times 8 \times 0.075$ cm) prepared and processed according to [19].

After electrophoresis, the gel was stained with a solution of Coomassie blue (2 mg/ml), ethanol (25%), and acetic acid (10%) for 1 h; rinsed with water (twice); and incubated for 1–2 h after the addition of a destaining solution (25% ethanol and 10% acetic acid).

Destained electrophoretograms were scanned with a DM-1 densitometer (Russia) over the protein fractions with molecular masses of 55, 30, 20, and 19 kDa. Optical densities were directly proportional to the protein concentrations; hence, this procedure provided us with objective estimates of quantitative variations in the protein composition of saliva (PCS). An example of the electrophoretic separation of salivary proteins is shown in Fig. 1.

Statistical analysis was performed using the Statistica for Windows v.4.3 software; the tables show all the data as the means and standard errors of the means ($M \pm m$). The significance of the differences between paired and unpaired groups was determined using Student's *t*-test. Correlations between the PCS and the psychological and autonomic indices were expressed as Spearman rank correlation coefficients.

RESULTS AND DISCUSSION

The human ability to endure (without serious consequences) stress strongly depends on the individual temperament, the specific qualities of the personality, and the personal experience in facing difficulties. In our previous work, the PCS of psychologically and emotionally stable subjects showed an above-normal percentage of 55-kDa proteins visible on electrophoretograms with numerous protein fractions, a picture not affected by various stress-producing factors [5]. Therefore, we concluded that the amount of these proteins (along with the sharpness of their electrophoretic bands) is an important indicator of human stress resistance.

The PCS determined before and after the model PES allowed us to divide the subjects into two groups.

Group 1 comprised seven subjects with a rather high resistance to stress. The optical densities of their 55–kDa proteins were above 0.11; the stress either did not affect this fraction or increased the amount of proteins 55–1 and 55–2.

In subjects with a low resistance to stress (group 2, $N = 17$), either no 55-kDa band was displayed at all or the 55-2 fraction could be decreased by stress. Figure 2

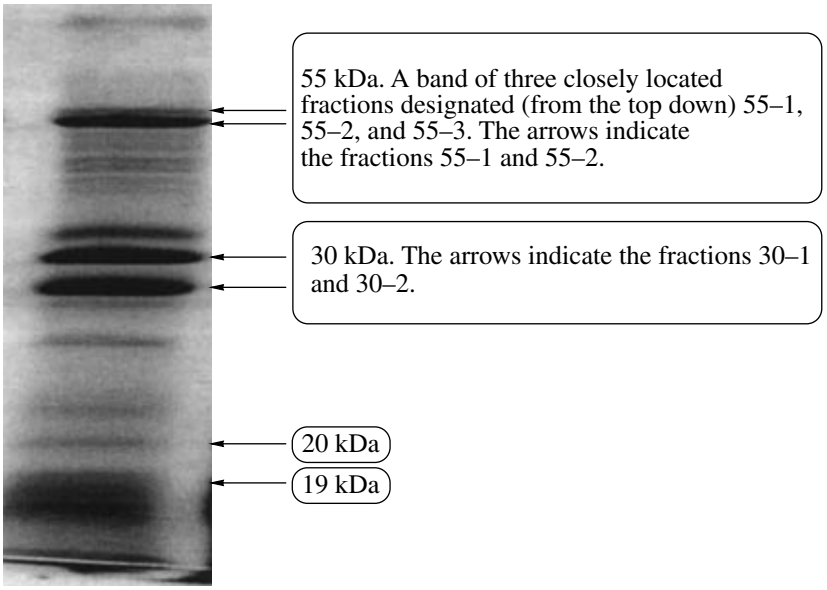

Fig. 1. An electrophoretogram of human salivary proteins.

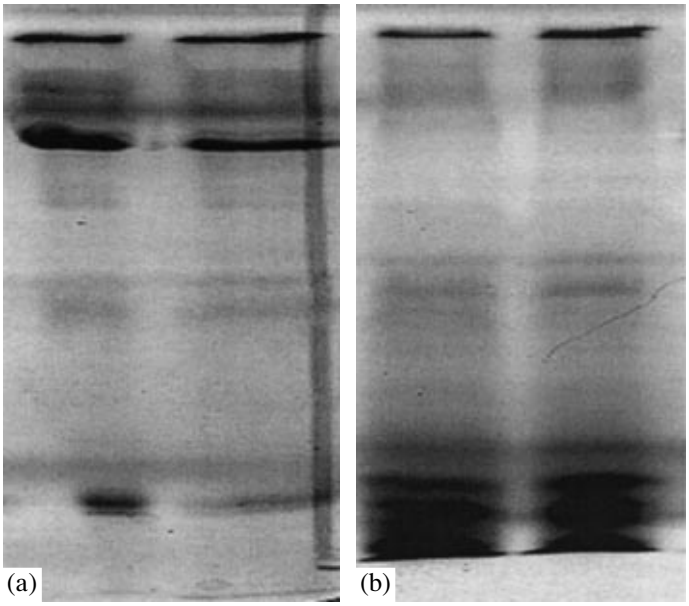

Fig. 2. Electrophoretograms typical of subjects with (a) high or (b) low stress resistance recorded (1) before or (2) after the model PES.

shows the most representative electrophoretograms of groups 1 and 2 recorded before and after the model stress.

The comparison of the groups with respect to HRV revealed considerable differences in the initial states and autonomic responses to the model PES (Table 1).

Group 2 (with a salivary protein spectrum typical of low resistance to stress) had significantly higher low-frequency and lower high-frequency components of the HRV spectrum; as a consequence, this group had a significantly higher LF/HF ratio. Stress decreased the parameters LF and LF/HF and significantly increased the VLF. This indicates that stress induced a stronger initial activation of the sympathoadrenal system and higher tension of the autonomic regulation; the increase in the VLF range suggests the involvement of suprasegmental autonomic regulatory centers.

Group 1 (with a predicted higher resistance to stress) had normal HRV parameters, and the model stress did not affect them.

Table 1. Initial and poststress autonomic parameters ($M \pm m$) of subjects with high or low (groups 1 and 2, respectively) stress resistance inferred from the changes in the PCS

Parameter	Group 1 ($N = 7$)		Group 2 ($N = 17$)	
	initial state	after the PES	initial state	after the PES
RMSSD, ms	48.94 ± 5.59	46.68 ± 3.66	296.51 ± 246.51	49.48 ± 5.30
pNN_{50}, %	37.72 ± 344.48	30.72 ± 4.99	19.03 ± 3.83**	18.83 ± 3.68**
CV, %	7.47 ± 0.82	7.08 ± 0.32	5.66 ± 16.74	6.82 ± 0.61
TP, ms^2 1000	2728.07 ± 708.46	3387.99 ± 524.61	2708.28 ± 688.98	3230.99 ± 522.59
LF/*HF*	1.21 ± 0.18	1.22 ± 0.25	4.14 ± 0.74**	2.39 ± 0.33*
VLF, %	29.89 ± 4.92	38.43 ± 8.44	30.34 ± 3.69	39.63 ± 3.18*
LF, %	36.15 ± 1.93	31.87 ± 5.05	53.73 ± 4.14**	35.67 ± 2.62*
HF, %	34.09 ± 4.81	29.68 ± 5.50	15.92 ± 2.01**	24.68 ± 3.71
TI, arb. units	51.18 ± 8.90	43.21 ± 2.90	58.95 ± 9.68	72.91 ± 11.99

Note: Here and in Tables 2–4: * significantly different from initial values ($p < 0.05$ or better); ** significant differences between groups; for abbreviations, see Methods.

Table 2. Mean values of the initial and poststress psychological indices ($M \pm m$) of subjects with high or low (groups 1 and 2, respectively) stress resistance inferred from the changes in the PCS

Parameter	Group 1 ($N = 7$)		Group 2 ($N = 17$)	
	initial state	after the PES	initial state	after the PES
LSC	54.71 ± 3.13	48.85 ± 3.47	50.82 ± 1.48	45.29 ± 1.73*
PEM	25.28 ± 2.64	24.71 ± 1.83	25.17 ± 0.91	22.76 ± 1.14*
NEM	13.00 ± 0.57	13 ± 0.65	15.88 ± 1.40	16.11 ± 1.39
ADEM	12.57 ± 1.50	12.28 ± 1.53	15.82 ± 1.83	15.52 ± 1.54
Interest	10.00 ± 1.15	9.00 ± 0.75	10.76 ± 0.53	8.35 ± 0.66*
Fear	3.57 ± 0.36	3.42 ± 0.29*	4.35 ± 0.41	3.70 ± 0.22
SAn	34.85 ± 2.51	37.14 ± 2.51	38.00 ± 1.56	43.23 ± 1.48*, **
SD	14.00 ± 0.78	15.14 ± 0.76	17.35 ± 0.74	18.94 ± 0.79*
SD/eu	16.00 ± 0.78	15.14 ± 0.76	13.94 ± 0.54	12.94 ± 0.66
SD/dys	5.00 ± 0.001	5.28 ± 0.28	6.76 ± 0.72	6.88 ± 0.41
SAg	16.28 ± 0.83	17.57 ± 1.60	16.47 ± 0.61	17.35 ± 0.73
SAg/e	5.00 ± 0.001	5.57 ± 0.29	5.58 ± 0.29	6.52 ± 0.42*
SAg/v	5.14 ± 0.14	5.57 ± 0.42	5.58 ± 0.27	5.64 ± 0.33
SAg/ph	6.14 ± 0.82	6.42 ± 1.10	5.29 ± 0.18	5.17 ± 0.09

The initial psychological characteristics of both groups were nearly identical. The groups, however, differed significantly in their reactions to stress (Table 2).

In contrast to group 1 (neither physically nor mentally affected by stress), the subjects of group 2 displayed, in addition to the above-mentioned decrease in the 55-kDa salivary protein content, a number of negative changes in their psychological indices: a decreased level of subjective comfort (LSC); apathy; and a parallel increase in the situational anxiety, situational depression, and SAg/e.

These changes in the psychological characteristics can be interpreted as mental strain with depressed mood, apathy, higher anxiety, irritability, and anger that can be provoked by any underestimation of the subject's cognitive activity.

Table 3. Number of significant correlations between the concentrations of salivary proteins and characteristics of the subjects' current mental and emotional states

Psychological characteristic of the current state	Before (1) or after (2) the stress test	Number of correlating parameters of the PCS	Molecular weights (kDa) of proteins with (+) positive or (–) negative correlations
Grief	2	6	(+) 30, 20
ADEM	1	6	(+) 30, 20
	2	8	(–) 55, (+) 30, 20, 19
Shame	1	8	(+) 30, 20, 19
	2	9	(+) 30, 20, 19
Guilt	1	9	(+) 30, 20
SAn	1	6	(+) 30, 20
SAg/v	1	9	(+) 30, 20, 19

Table 4. Number of significant correlations between the concentrations of salivary proteins and characteristics of the subjects' autonomic status

HRV parameter	Before (1) or after (2) the stress test	Number of correlating parameters of the PCS	Molecular weights (kDa) of proteins with (+) positive or (–) negative correlations
pNN_{50}	1	7	(–) 55–3, 30, 20
	2	7	(–) 55–3, 30, 20
SDNN	1	1	(+) 19
	2	1	
CV	1	1	(+) 19
LF/*HF*	1	2	(–) 55–2, (+) 19
	2	1	(+) 55–3
LF	1	3	(+) 30
HF	1	2	(+) 55–2
	2	–	(+) 19
HR	1	7	(+) 55–3, 30, 20
	2	7	(+) 55–3, 30, 20
Mo	1	7	(+) 55–3, 30, 20
	2	4	(+) 30
AMo	2	7	(+) 30, 20, 19
ΔX	1	2	(–) 55–3, 30–2
	2	1	(–) 55–3
TI	1	7	(+) 55–3, 30, 20
	2	7	(+) 55–3, 30, 20

Hence, the initial salivary protein spectrum and its changes were correlated with autonomic and mental changes caused by the model stress: as a response to stress, the subjects with a decreased 55-kDa salivary protein content (which can be further decreased by stress) developed a highly pronounced syndrome of mental strain accompanied by the activation of autonomic regulatory mechanisms.

We found 76 significant correlations ($r \geq 0.40$) between quantitative parameters of the PCS and psychological indices.

The strongest correlations were observed between nine different parameters of the PCS and the changes in the psychological characteristics of the current mental and emotional state, such as the poststress emotion of shame, the prestress emotion of guilt, and verbal manifestations of anger. Table 3 shows that three electrophoretic fractions (fractions 1 and 2 within the 30-kDa range and the 20-kDa fraction) were most sensitive to the mental and emotional changes caused by the PES (the higher salivary concentrations of these proteins, the stronger stress-induced emotions of anger, shame, fear, anxiety, and depression).

In addition, we found 75 significant correlations between salivary protein fractions and parameters of HRV that reflected the autonomic status of the subjects (Table 4).

The strongest and most numerous correlations were observed between seven different protein fractions and the pNN_{50}, HR, TI (both before and after the psychological cognitive stress test), prestress Mo, and posttest AMo. This high correlation suggests the involvement of the autonomic nervous system in the regulation of the PCS; in other words, these data demonstrate that the PCS is sensitive to the actual autonomic status and to changes in the mental and emotional states. Some of the correlations between the PCS and autonomic parameters demonstrated a parallel increase in the extent of the sympathetic activation caused by the model PES (manifested through the changes in the AMo, HR, and LF) and in the concentrations of the 20- and 30-kDa proteins (especially, in the concentration of the protein 30–2). Changes in the parasympathetic activity (i.e., changes in the pNN_{50}, HF, and ΔX) were inversely proportional to the concentrations of these relatively low-molecular-weight fractions; however, they were directly proportional to the concentration of the protein 55–2.

CONCLUSIONS

Changes in the protein composition of total saliva induced by model psychological stress and the correlations between the concentrations of individual proteins and psychological and autonomic characteristics indicate that this composition is sensitive to the subject's mental and emotional state.

Low initial concentrations of the salivary protein 55–2 and their further poststress decrease, as well as high initial concentrations of 20- and 30-kDa proteins (especially of the protein 30–2) and their poststress increase, could serve as indicators of a low resistance to psychological stress.

To date, we are not absolutely sure that the concentrations of salivary proteins provide correct prognostic assessments of individual stress resistance. Nevertheless, we believe that this test deserves further verification as a promising noninvasive test that could be helpful in the complex assessment of the subject's mental and emotional state and in predicting individual resistance to environmental stress.

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